

# WHAT IS CLAIMED IS:

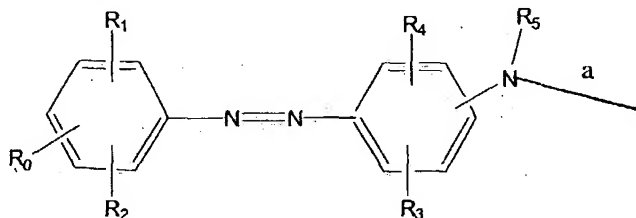
1. An oligonucleotide conjugate having the formula



where ODN is an oligonucleotide or nucleic acid;

FL is a fluorophore moiety covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, and

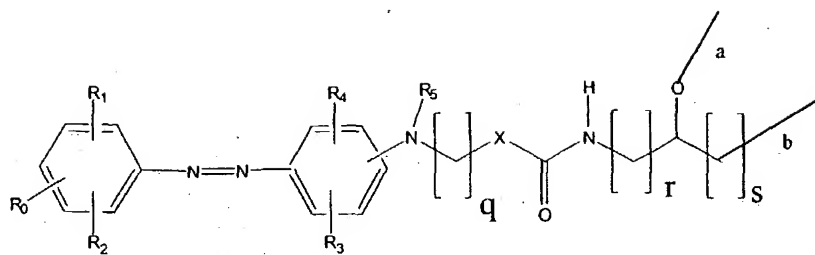
Q is a quencher moiety covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, the quencher moiety having the structure



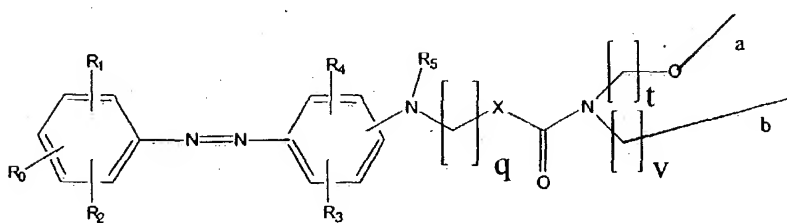
where R<sub>0</sub>, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are independently -H, halogen, -O(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>, -(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub> where n=0 to 5, -NO<sub>2</sub>, -SO<sub>3</sub>, -N[(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>]<sub>2</sub> where n'=0 to 5 or -CN, and R<sub>5</sub>=-H or -(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub> where n''=0 to 5, and where the quencher moiety is attached to the linker through the valence bond designated a.

2. An oligonucleotide conjugate in accordance with Claim 1 where R<sub>0</sub> is H, R<sub>1</sub> is NO<sub>2</sub> in the 4 position of the benzene nucleus, R<sub>2</sub> is H or Cl in the 2 position of the benzene nucleus, and R<sub>3</sub> and R<sub>4</sub> are hydrogen and R<sub>5</sub> is ethyl.

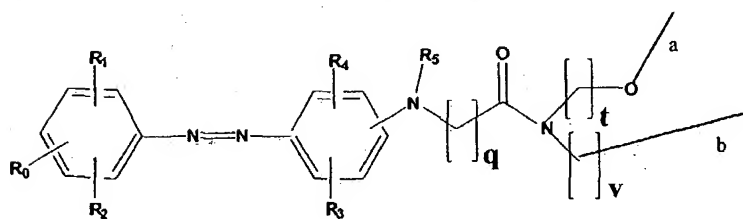
3. An oligonucleotide conjugate in accordance with Claim 1 where the quencher moiety and the linker attaching it to the ODN comprises the structures selected from the moieties shown by the formulas Q-1, Q-2 and Q-3



Q-1



Q-2

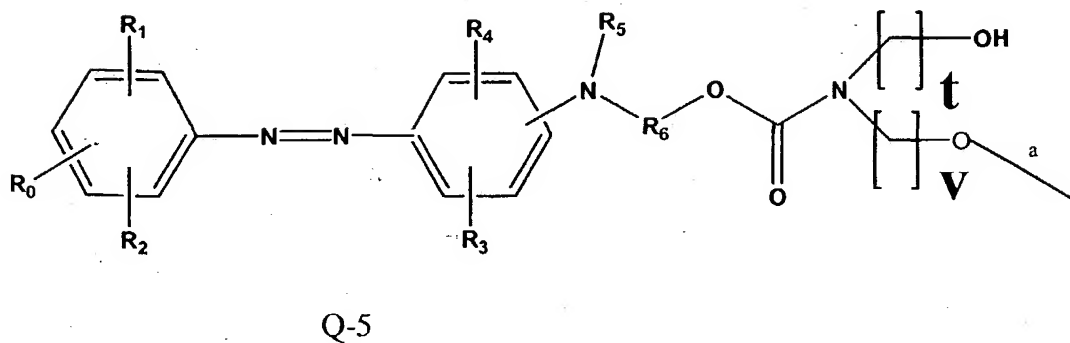
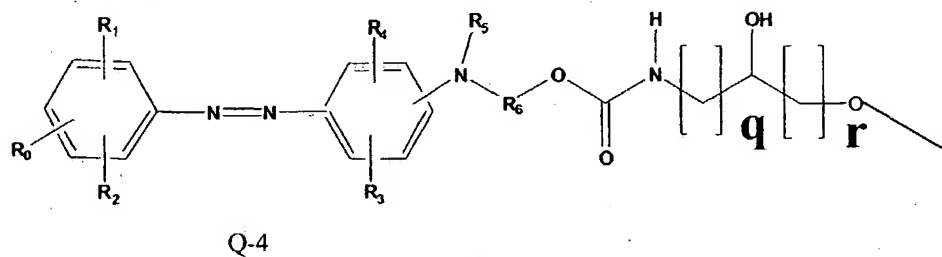


Q-3

where  $q$  is 1 to 20,  $X$  is  $-O-$ ,  $-OCH_2-$  or  $-CH_2-$ ;  $t$  and  $v$  independently are 1 to 20,  $r$  and  $s$  independently are 1 to 20, and the conjugated quencher and linker moiety is attached to the ODN through one of the valence bonds designated  $a$  or  $b$ .

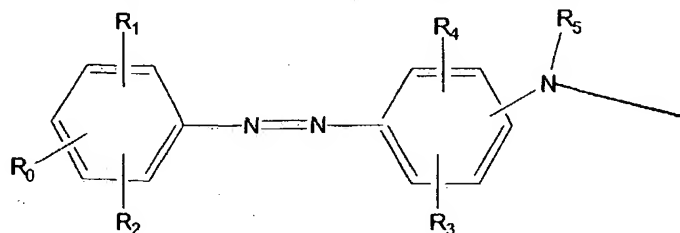
4. An oligonucleotide conjugate in accordance with Claim 3 further comprising a minor groove binder moiety attached to the quencher-linker conjugate through one of the valence bonds designated  $a$  or  $b$ .

5. An oligonucleotide conjugate in accordance with Claim 1 where the quencher moiety and of the linker attaching it to the ODN comprises the structures selected from the moieties shown by the formulas Q-4, and Q-5



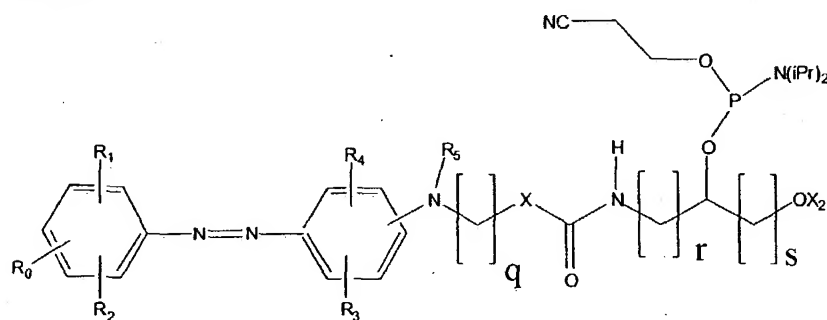
where  $R_6$  is  $-(CH_2)_{n^*}$  where  $n^*$  is 1 to 20, and  $t$  and  $v$  independently are 1 to 20, and where the quencher moiety is attached to the ODN through the valence bond designated  $a$ .

6. A phosphoramidite reagent for preparing an oligonucleotide-fluorophore-quencher conjugate, the reagent including the moiety

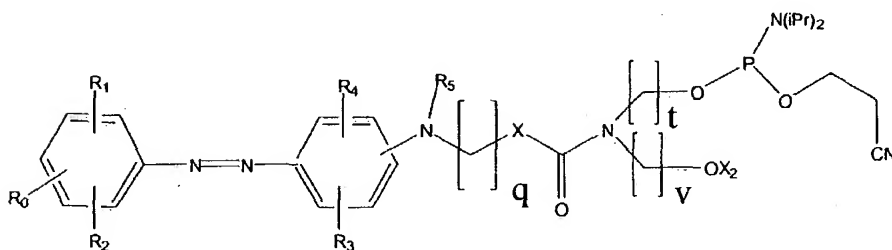


where  $R_0, R_1, R_2, R_3$  and  $R_4$  are independently  $-H$ , halogen,  $-O(CH_2)_nCH_3$ ,  $-(CH_2)_nCH_3$  where  $n = 0$  to  $5$ ,  $-NO_2$ ,  $-SO_3$ ,  $-N[(CH_2)_nCH_3]_2$  where  $n' = 0$  to  $5$  or  $-CN$ , and  $R_5 = -H$  or  $-(CH_2)_nCH_3$  where  $n'' = 0$  to  $5$ , and a bis(methylethyl)amino](2-cyanoethoxy)phosphinoxy moiety covalently linked thereto.

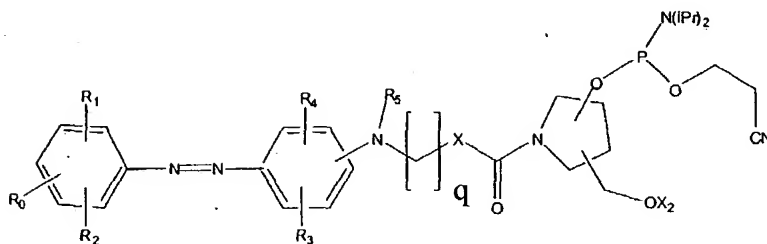
7. A phosphoramidite reagent in accordance with Claim 6 having the formula selected from the group consisting of the formulas designated PA-1, PA-2 and PA-3



PA-1



PA-2



PA-3

where  $R_0, R_1, R_2, R_3$  and  $R_4$  are independently  $-H$ , halogen,  $-O(CH_2)_nCH_3$ ,  $-(CH_2)_nCH_3$  where  $n = 0$  to  $5$ ,  $-NO_2$ ,  $-SO_3$ ,  $-N[(CH_2)_nCH_3]_2$  where  $n' = 0$  to  $5$  or  $-CN$ , and  $R_5 = -H$  or  $-(CH_2)_nCH_3$  where  $n'' = 0$  to  $5$ ,  $q$  is 1

to 20, X is -O- or -CH<sub>2</sub>-; t, v, r and s independently are 1 to 20, and X<sub>2</sub> is H or dimethoxytrityl, methoxytrityl, trityl or an acid labile blocking group.

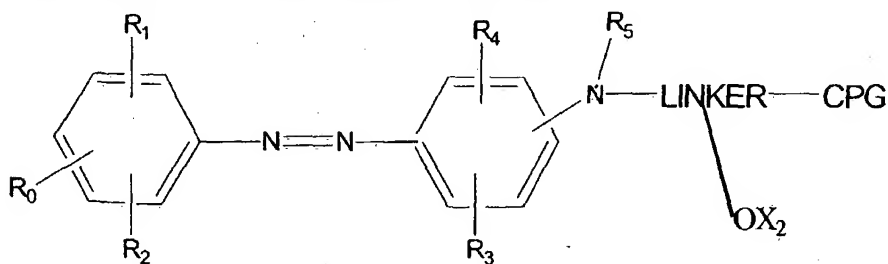
8. A phosphoramidite reagent in accordance with Claim 7 that has the formula designated PA-1.

9. A phosphoramidite reagent in accordance with Claim 7 that has the formula designated PA-2.

10. A phosphoramidite reagent in accordance with Claim 7 that has the formula designated PA-3.

11. A phosphoramidite reagent in accordance with Claim 7 where R<sub>0</sub> is H, R<sub>1</sub> is NO<sub>2</sub> in the 4 position of the benzene nucleus, R<sub>2</sub> is Cl in the 2 position of the benzene nucleus, and R<sub>3</sub> and R<sub>4</sub> are hydrogen and R<sub>5</sub> is ethyl.

12. A covalently linked solid support and quencher conjugate suitable for oligonucleotide synthesis, having the structure



where CPG stands for a polymeric solid support;

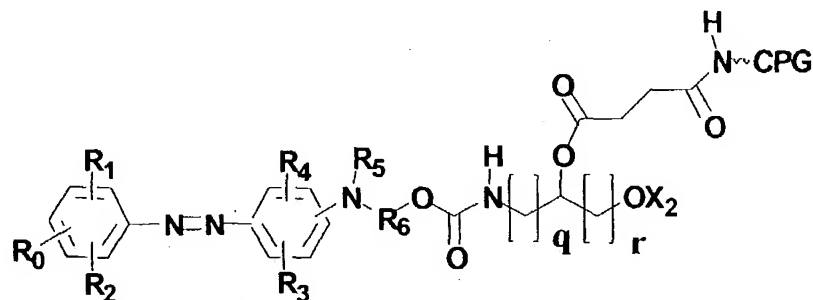
LINKER is a moiety having the length of 1 to approximately 30 atoms and linking the diphenylazo moiety to the CPG;

X<sub>2</sub> is OH or , dimethoxytrityl, methoxytrityl, trityl or an acid labile blocking group;

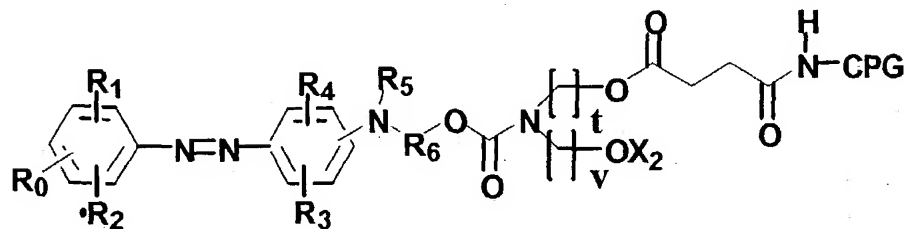
R<sub>0</sub>, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are independently -H, halogen, -O(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>,

$-(CH_2)_nCH_3$  where  $n = 0$  to  $5$ ,  $-NO_2$ ,  $-SO_3$ ,  $-N[(CH_2)_nCH_3]_2$  where  $n' = 0$  to  $5$  or  $-CN$ , and  $R_5 = -H$  or  $-(CH_2)_nCH_3$  where  $n'' = 0$  to  $5$ .

13. A covalently linked solid support and quencher conjugate in accordance with Claim 12 selected from the structures



and



where  $R_6$  is  $-(CH_2)_{n^*}$  where  $n^*$  is  $1$  to  $20$ , and  $q$ ,  $r$ ,  $t$  and  $v$  independently are  $1$  to  $20$ .

14. A covalently linked solid support and quencher conjugate in accordance with Claim 13 where  $R_0$  is  $H$ ,  $R_1$  is  $NO_2$  in the 4 position of the benzene nucleus,  $R_2$  is  $Cl$  in the 2 position of the benzene nucleus, and  $R_5$  is ethyl.

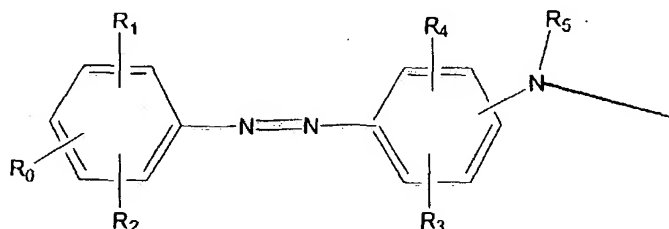
15. An oligonucleotide conjugate having the formula

**FL-ODN-Q-MGB**

where ODN is an oligonucleotide or nucleic acid;

FL is a fluorophore covalently attached to the ODN through a linker having the length of  $0$  to approximately  $30$  atoms, and

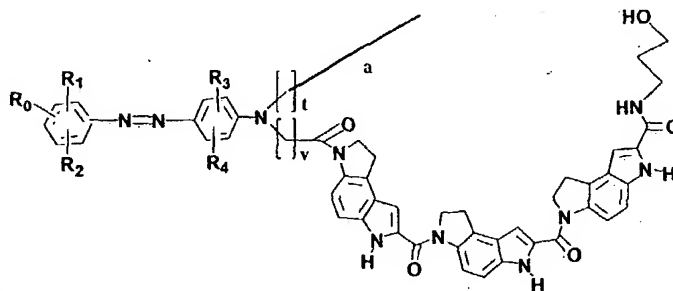
Q is a quencher moiety covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, the quencher moiety having the structure



where  $R_0$ ,  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are independently  $-H$ , halogen,  $-O(CH_2)_nCH_3$ ,  $-(CH_2)_nCH_3$  where  $n = 0$  to  $5$ ,  $-NO_2$ ,  $-SO_3$ ,  $-N[(CH_2)_{n'}CH_3]_2$  where  $n' = 0$  to  $5$  or  $-CN$ , and  $R_5 = -H$ ,  $-(CH_2)_{n''}CH_3$  or  $-(CH_2)_{n''}$  where  $n'' = 0$  to  $5$ , and

MGB is minor groove binder moiety covalently attached to the ODN moiety or to the quencher moiety through a linker having the length of 0 to approximately 30 atoms.

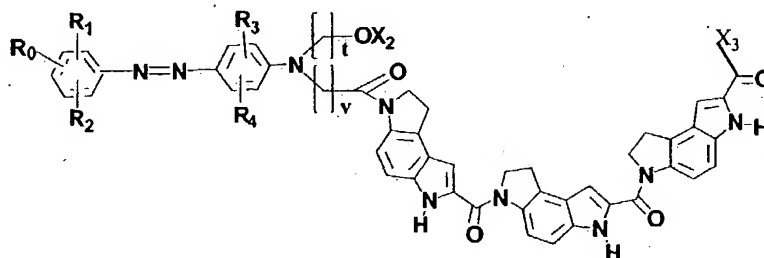
16. An oligonucleotide conjugate in accordance with Claim 15 where the MGB moiety is attached to the quencher moiety, and the covalently bonded MGB-Q moiety has the structure



where  $t$  and  $v$  independently are 1 to 20, and the valence bond designated **a** attaches the MGB-Q moiety to the ODN moiety.

17. An oligonucleotide conjugate in accordance with Claim 16 where  $R_0$  is  $H$ ,  $R_1$  is  $NO_2$  in the 4 position of the benzene nucleus,  $R_2$  is  $H$  or  $Cl$  in the 2 position of the benzene nucleus, and  $R_3$  and  $R_4$  are hydrogen.

18. A covalently bonded minor groove binder and quencher reagent for oligonucleotide synthesis, having the formula



where  $R_0$ ,  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are independently -H, halogen,  $-O(CH_2)_nCH_3$ ,  $-(CH_2)_nCH_3$  where  $n = 0$  to  $5$ ,  $-NO_2$ ,  $-SO_3$ ,  $-N[(CH_2)_nCH_3]_2$  where  $n' = 0$  to  $5$  or  $-CN$ , and  $t$  and  $v$  independently are  $1$  to  $20$ ;

$X_2$  is H or dimethoxytrityl, methoxytrityl, trityl or an acid labile blocking group, and

$X_3$  is pentafluorophenyl, or NH-LINKER-CPG or O-LINKER-CPG where CPG is a polymeric solid support and LINKER is a linking moiety having a length of approximately  $0$  to  $30$  atoms linking the tricyclic moiety to the CPG.

19. A covalently bonded minor groove binder and quencher reagent in accordance with Claim 18 wherein  $X_3$  is pentafluorophenyl.

20. A covalently bonded minor groove binder and quencher reagent in accordance with Claim 18 wherein  $X_3$  is NH-LINKER-CPG or O-LINKER-CPG.

21. A covalently bonded minor groove binder and quencher reagent in accordance with Claim 18 where  $R_0$  is H,  $R_1$  is  $NO_2$  in the 4 position of the benzene nucleus,  $R_2$  is H or Cl in the 2 position of the benzene nucleus,  $R_3$  and  $R_4$  are hydrogen and  $v=t=3$ .



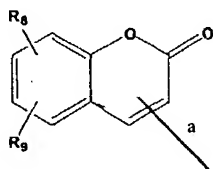
22. An oligonucleotide conjugate having the formula

**FL-ODN-Q**

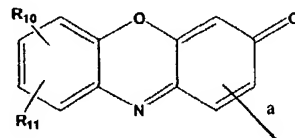
where ODN is an oligonucleotide or nucleic acid;

Q is a quencher moiety covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, and

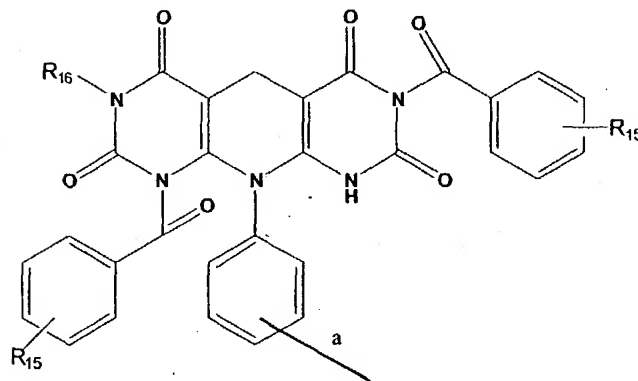
FL is a fluorophore covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, said fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein  $R_8$  and  $R_9$  independently are H, halogen,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{C}(=\text{O})\text{NH}_2$ , or  $-\text{CN}$ ;  $-\text{OR}_{nn}$ ,  $-\text{SR}_{nn}$ ,  $-\text{OR}_{nn}$ ,  $-\text{NHR}_{nn}$ ,  $-\text{N}[\text{R}_{nn}]_2$  where  $\text{R}_{nn}$  is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

$R_{10}$  and  $R_{11}$  independently are H,  $-\text{CN}$ ,  $-\text{OR}_{12}$ ,  $-\text{N}(\text{R}_{12})_2$ , halogen,  $-\text{O}(\text{CH}_2)_n\text{CH}_3$ ,  $-(\text{CH}_2)_n\text{CH}_3$ ,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{C}(=\text{O})\text{NH}_2$ ,  $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$  where  $n=0$  to 5, or  $\text{R}_{12}$  is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

$\text{R}_{15}$  is H or alkyl of 1 to 10 carbons;

$\text{R}_{16}$  is alkyl of 1 to 10 carbons, and

the valence bond designated a symbolizes covalent attachment of the fluorophore to the linker.

23. An oligonucleotide conjugate in accordance with Claim 22 where the fluorophore has the formula designated FL-1.

24. An oligonucleotide conjugate in accordance with Claim 23 where  $R_8$  is  $OC(O)CH(CH_3)_2$  and  $R_9$  is H.

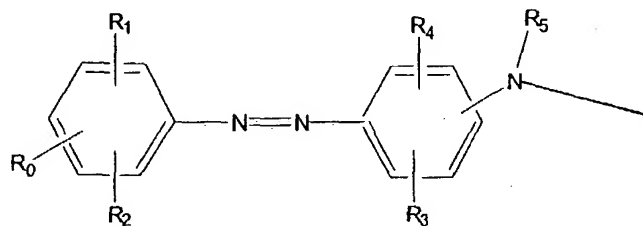
25. An oligonucleotide conjugate in accordance with Claim 22 where the fluorophore has the formula designated FL-2.

26. An oligonucleotide conjugate in accordance with Claim 25 where  $R_{10}$  is  $OC(O)CH(CH_3)_2$  and  $R_{11}$  is H.

27. An oligonucleotide conjugate in accordance with Claim 22 where the fluorophore has the formula designated FL-3.

28. An oligonucleotide conjugate in accordance with Claim 28 where  $R_{15}$  is methyl and  $R_{16}$  is *n*-propyl.

29. An oligonucleotide conjugate in accordance with Claim 22 where the quencher moiety comprises the structure

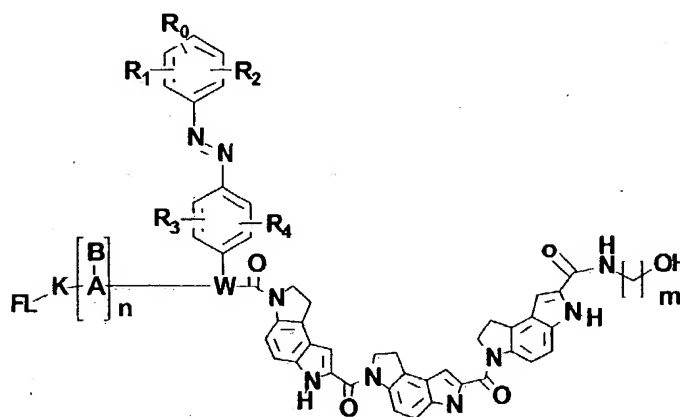


where  $R_0$ ,  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are independently -H, halogen,  $-O(CH_2)_nCH_3$ ,  $-(CH_2)_nCH_3$  where  $n=0$  to 5,  $-NO_2$ ,  $-SO_3$ ,  $-N[(CH_2)_nCH_3]_2$  where  $n'=0$  to 5 or  $-CN$ , and  $R_5 = -H$  or  $-(CH_2)_nCH_3$  where  $n''=0$  to 5.

30. An oligonucleotide conjugate in accordance with Claim 22 comprising an additional minor groove binder moiety (MGB) attached to the quencher moiety through a linker having the length of 0 to approximately 30 atoms, whereby the oligonucleotide conjugate has the formula



31. An oligonucleotide conjugate of the formula



wherein  $R_0$ ,  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are independently -H, halogen,  $-O(CH_2)_{n^*}CH_3$ ,  $-(CH_2)_{n^*}CH_3$  where  $n^*=0$  to 5,  $-NO_2$ ,  $-SO_3$ ,  $-N[(CH_2)_{n^*}CH_3]_2$  where  $n^*=0$  to 5 or  $-CN$ ;

FL is a fluorophore moiety with emission wavelengths in the range of about 300 to about 800 nm;

K is a linker containing 1 to approximately 30 atoms selected from the group consisting of C, O, N, S, P and H;

$[A-B]_n$  symbolizes an ODN, DNA, RNA or PNA or any combination thereof, where A is the sugar phosphate backbone where the sugar and the phosphate may independently be modified; B is a heterocyclic base, where B is independently selected from purine, pyrimidine, pyrazolo[3,4-d]pyrimidine, 7-substituted pyrazolo[3,4-d]pyrimidine-, 7-deazapurine, 7-substituted 7-

deazapurine, and modified purine- and pyrimidine-bases, and where the DNA, RNA, PNA or ODN can include any combinations of these bases, and

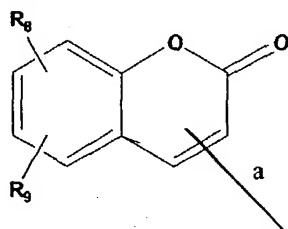
and **n** is the number of nucleotide units in said DNA, RNA, PNA or ODN;

**W** is a linker of a length of 0 to approximately 30 atoms, selected from the group consisting of C, O, N, S, P and H, and

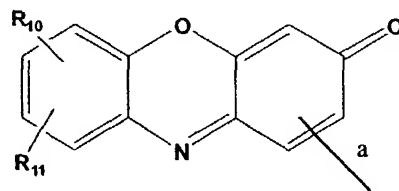
**m** is an integer having the values of 1 to 20.

32. An oligonucleotide conjugate in accordance with Claim 31 where  $R_0$  is H,  $R_1$  is  $NO_2$  in the 4 position of the benzene nucleus,  $R_2$  is H or Cl in the 2 position of the benzene nucleus, and  $R_3$  and  $R_4$  are hydrogen.

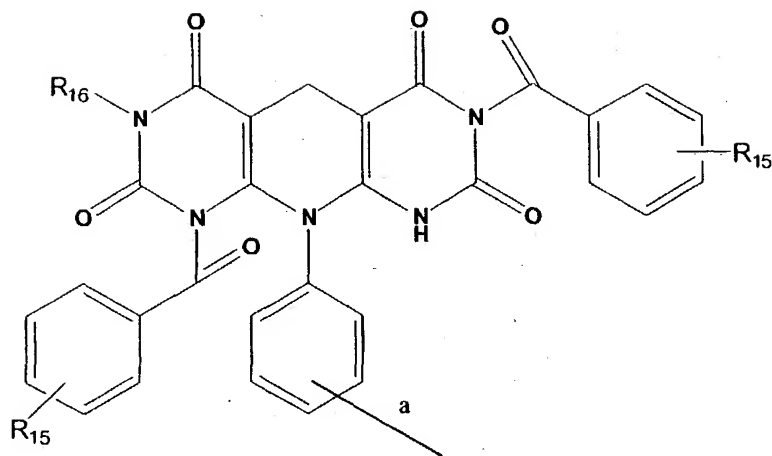
33. An oligonucleotide conjugate in accordance with Claim 31 where said fluorophore moiety has the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

where  $R_8$  is OH or O-alkanoyl where the alkanoyl group has 1 to 10 carbons;

$R_9$  is H or alkyl of 1 to 10 carbons;

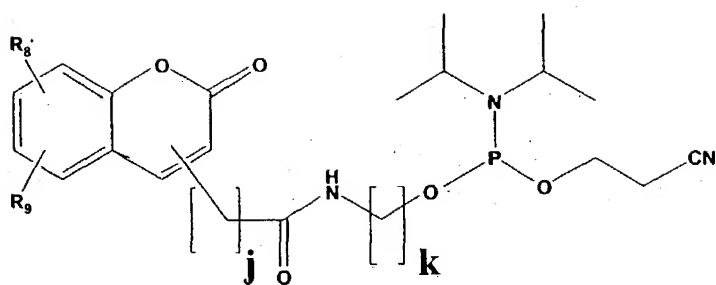
$R_{10}$  and  $R_{11}$  independently are H,  $-OR_{12}$ ,  $-NHR_{13}$ , halogen,  $-O(CH_2)_nCH_3$ ,  $-(CH_2)_nCH_3$ ,  $-NO_2$ ,  $-SO_3$ ,  $-C(=O)NH_2$ ,  $-N[(CH_2)_nCH_3]_2$  or  $-CN$  where  $n=0$  to 5;

$R_{15}$  is H or alkyl of 1 to 10 carbons;

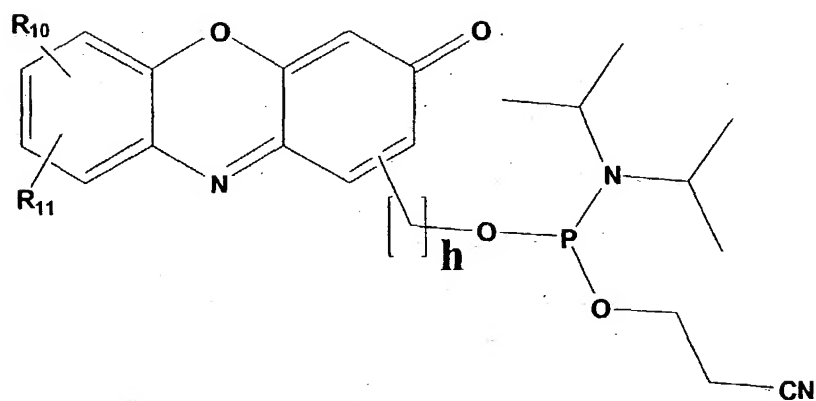
$R_{16}$  is alkyl of 1 to 10 carbons, and

the valence bond designated a symbolizes covalent attachment of the fluorophore to the linker K.

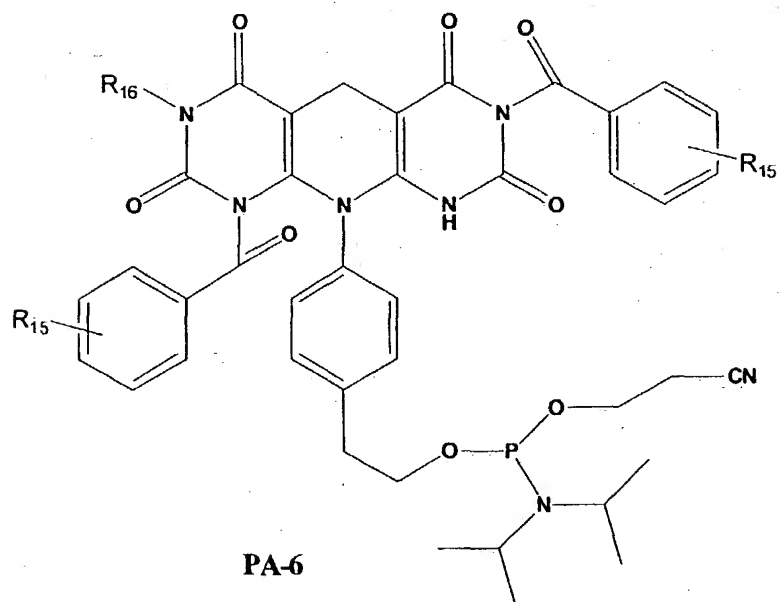
34. A phosphoramidite reagent for preparing an oligonucleotide-fluorophore-quencher conjugate, the reagent selected from the group consisting of the structures designated PA-4, PA-5 and PA-6,



PA-4



PA-5



PA-6

wherein  $R_8$  and  $R_9$  independently are H, halogen,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{C}(=\text{O})\text{NH}_2$ , or  $-\text{CN}$ ;  $-\text{OR}_{nn}$ ,  $-\text{SR}_{nn}$ ,  $-\text{OR}_{nn}$ ,  $-\text{NHR}_{nn}$ ,  $-\text{N}[\text{R}_{nn}]_2$  where  $R_{nn}$  is independently H, a blocking group compatible with oligomer synthesis

removable under acid or alkaline conditions; or an alkyl or alkanoyl group having 1 to 10 carbon atoms;

j and k independently are 1 to 10;

$R_{10}$  and  $R_{11}$  independently are H,  $-OR_{12}$ ,  $-NHR_{13}$ , halogen,  $-O(CH_2)_nCH_3$ ,  $-(CH_2)_nCH_3$ ,  $-NO_2$ ,  $-SO_3$ ,  $-C(=O)NH_2$ ,  $-N[(CH_2)_nCH_3]_2$ , O-alkyl or O-alkanoyl where the alkanoyl group has 1 to 10 carbons, or  $-CN$  where  $n=0$  to 5;  $h=1$  to 20; and  $R_{12}$  and  $R_{13}$  are blocking groups compatible with ODN synthesis;

$R_{15}$  is H or alkyl of 1 to 10 carbons;

$R_{16}$  is alkyl of 1 to 10 carbons.

35. A phosphoramidite reagent in accordance with Claim 34 that has the formula designated PA-4.

36. A phosphoramidite reagent in accordance with Claim 35 where  $R_8$  is  $-OC(O)CH(CH_3)_2$ ,  $R_9$  is H, j is 2 and k is 6.

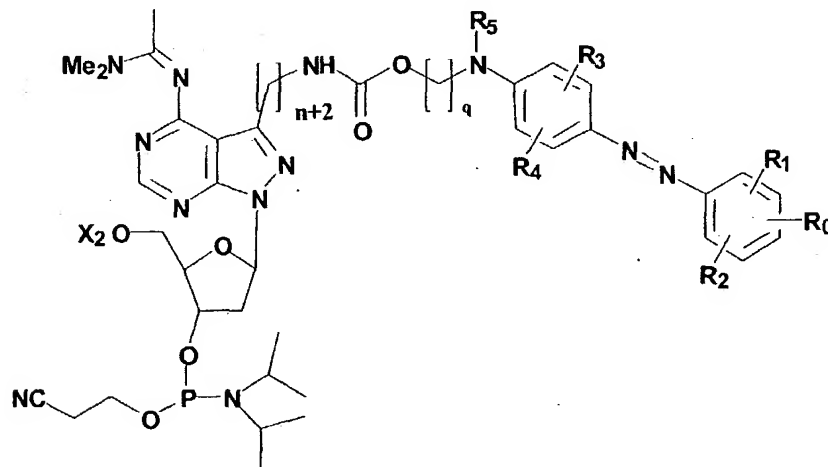
37. A phosphoramidite reagent in accordance with Claim 34 that has the formula designated PA-5.

38. A phosphoramidite reagent in accordance with Claim 37 where  $R_{10}$  is  $OC(O)CH(CH_3)_2$ ,  $R_{11}$  is H and h is 3.

39. A phosphoramidite reagent in accordance with Claim 34 that has the formula designated PA-6.

40. A phosphoramidite reagent in accordance with Claim 39 where  $R_{15}$  is methyl and  $R_{16}$  is *n*-propyl.

41. A phosphoramidite reagent for preparing an oligonucleotide-fluorophore-quencher conjugate, the reagent having the formula



wherein  $R_0$ ,  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are independently -H, halogen,  $-O(CH_2)_{n^*}CH_3$ ,  $-(CH_2)_{n^*}CH_3$  where  $n^* = 0$  to 5,  $-NO_2$ ,  $-SO_3$ ,  $-N[(CH_2)_{n^*}CH_3]_2$  where  $n^* = 0$  to 5 or  $-CN$ , and  $R_5 = -H$  or  $-(CH_2)_{n''}CH_3$  where  $n'' = 0$  to 5;

$n$  is 1 to 10;

$q$  is 1 to 20, and

$X_2$  is H or dimethoxytrityl, methoxytrityl, trityl or an acid labile blocking group.

42. A phosphoramidite reagent in accordance with Claim 41 where  $R_0$  is H,  $R_1$  is  $NO_2$  in the 4 position of the benzene nucleus,  $R_2$  is Cl in the 2 position of the benzene nucleus, and  $R_3$  and  $R_4$  are hydrogen,  $R_5$  is ethyl,  $n$  is 1 and  $q$  is 2.

43. An oligonucleotide conjugate having the formula

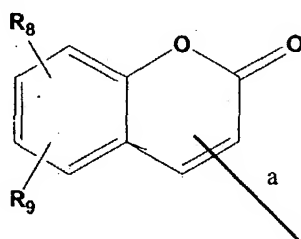
#### FL-ODN-MGB

where ODN is an oligonucleotide or nucleic acid;

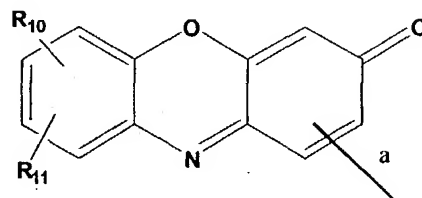
MGB is minor groove binder moiety covalently attached to the ODN moiety or to the quencher moiety through a linker having the length of 0 to approximately 30 atoms;



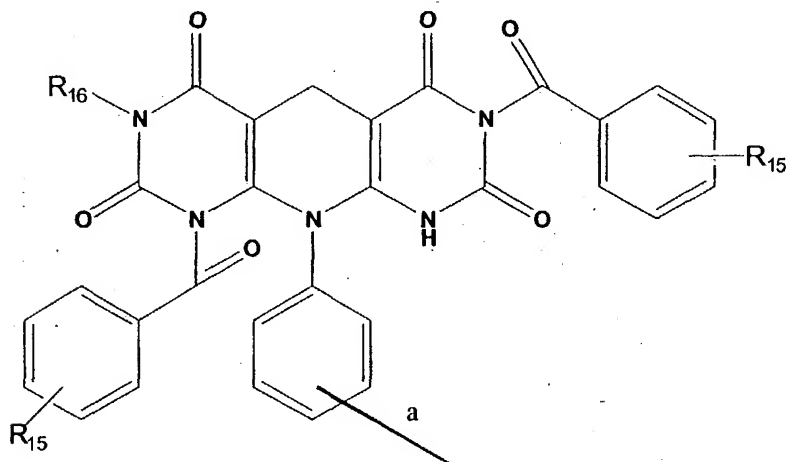
FL is a fluorophore covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, said fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein  $R_8$  and  $R_9$  independently are H, halogen,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{C}(=\text{O})\text{NH}_2$ , or  $-\text{CN}$ ;  $-\text{OR}_{nn}$ ,  $-\text{SR}_{nn}$ ,  $-\text{OR}_{nn}$ ,  $-\text{NHR}_{nn}$ ,  $-\text{N}[\text{R}_{nn}]_2$  where  $\text{R}_{nn}$  is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

$R_{10}$  and  $R_{11}$  independently are H,  $-\text{CN}$ ,  $-\text{OR}_{12}$ ,  $-\text{N}(\text{R}_{12})_2$ , halogen,  $-\text{O}(\text{CH}_2)_n\text{CH}_3$ ,  $-(\text{CH}_2)_n\text{CH}_3$ ,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{C}(=\text{O})\text{NH}_2$ ,  $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$  where  $n = 0$  to 5, or  $R_{12}$  is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

$R_{15}$  is H or alkyl of 1 to 10 carbons;

$R_{16}$  is alkyl of 1 to 10 carbons, and

the valence bond designated **a** symbolizes covalent attachment of the fluorophore to the linker.

44. An oligonucleotide conjugate in accordance with Claim 43 where the fluorophore has the formula designated FL-1.

45. An oligonucleotide conjugate in accordance with Claim 44 where  $R_8$  is  $-\text{OC}(\text{O})\text{CH}(\text{CH}_3)_2$  and  $R_9$  is H.

46. An oligonucleotide conjugate in accordance with Claim 43 where the fluorophore has the formula designated FL-2.

47. An oligonucleotide conjugate in accordance with Claim 46 where  $R_{10}$  is  $\text{OC}(\text{O})\text{CH}(\text{CH}_3)_2$  and  $R_{11}$  is H.

48. An oligonucleotide conjugate in accordance with Claim 43 where the fluorophore has the formula designated FL-3.

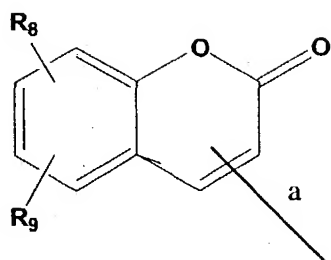
49. An oligonucleotide conjugate in accordance with Claim 49 where  $R_{15}$  is methyl and  $R_{16}$  is *n*-propyl.

50. An oligonucleotide conjugate having the formula

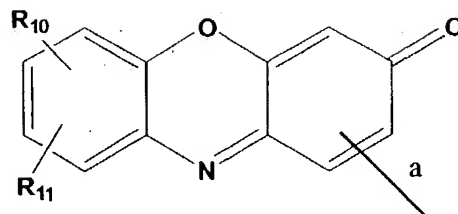
**FL-ODN**

where ODN is an oligonucleotide or nucleic acid;

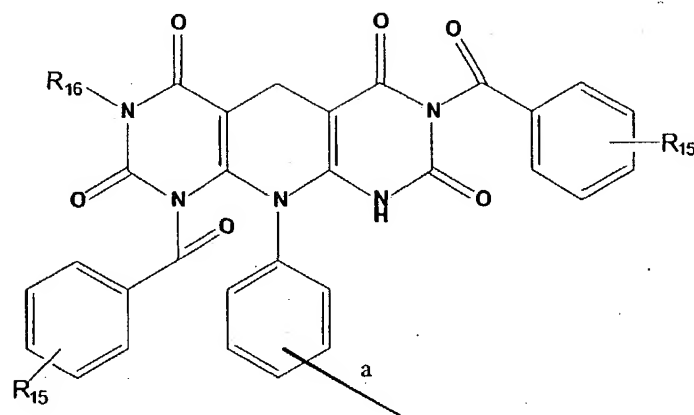
FL is a fluorophore covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, said fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein  $R_8$  and  $R_9$  independently are H, halogen,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{C}(=\text{O})\text{NH}_2$ , or  $-\text{CN}$ ;  $-\text{OR}_{nn}$ ,  $-\text{SR}_{nn}$ ,  $-\text{OR}_{nn}$ ,  $-\text{NHR}_{nn}$ ,  $-\text{N}[\text{R}_{nn}]_2$  where  $R_{nn}$  is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

$R_{10}$  and  $R_{11}$  independently are H,  $-\text{CN}$ ,  $-\text{OR}_{12}$ ,  $-\text{N}(\text{R}_{12})_2$ , halogen,  $-\text{O}(\text{CH}_2)_n\text{CH}_3$ ,  $-(\text{CH}_2)_n\text{CH}_3$ ,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{C}(=\text{O})\text{NH}_2$ ,  $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$  where  $n = 0$  to 5, or  $R_{12}$  is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

$R_{15}$  is H or alkyl of 1 to 10 carbons;

$R_{16}$  is alkyl of 1 to 10 carbons, and

the valence bond designated **a** symbolizes covalent attachment of the fluorophore to the linker.

51. An oligonucleotide conjugate in accordance with Claim 50 where the fluorophore has the formula designated FL-1.

52. An oligonucleotide conjugate in accordance with Claim 51 where  $R_8$  is  $OC(O)CH(CH_3)_2$  and  $R_9$  is H.

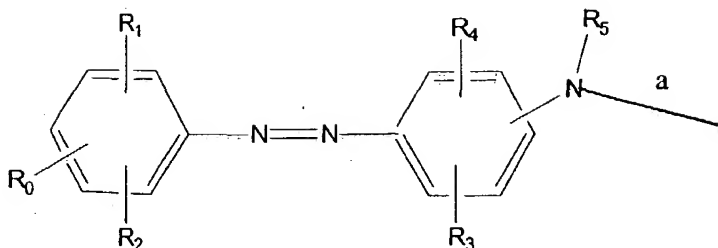
53. An oligonucleotide conjugate in accordance with Claim 50 where the fluorophore has the formula designated FL-2.

54. An oligonucleotide conjugate in accordance with Claim 53 where  $R_{10}$  is  $OC(O)CH(CH_3)_2$  and  $R_{11}$  is H.

55. An oligonucleotide conjugate in accordance with Claim 50 where the fluorophore has the formula designated FL-3.

56. An oligonucleotide conjugate in accordance with Claim 55 where  $R_{15}$  is methyl and  $R_{16}$  is *n*-propyl.

57. A method for hybridizing nucleic acids, comprising the steps of:  
(a) providing a first nucleic acid and a second nucleic acid,  
(b) incubating the nucleic acids under hybridization conditions, and  
(c) identifying hybridized nucleic acids; wherein at least one of the nucleic acids comprises a **FL-nucleic-acid-Q** conjugate where **FL** is a fluorophore moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms,, and **Q** is a quencher moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms, the quencher moiety having the structure

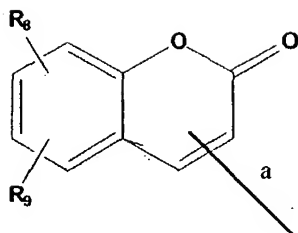


where  $R_0$ ,  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are independently -H, halogen,  $-\text{O}(\text{CH}_2)_n\text{CH}_3$ ,  $-(\text{CH}_2)_n\text{CH}_3$  where  $n=0$  to 5,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$  where  $n'=0$  to 5 or  $-\text{CN}$ , and  $R_5 = -\text{H}$  or  $-(\text{CH}_2)_n\text{CH}_3$  where  $n''=0$  to 5, and where the quencher moiety is attached to the linker through the valence bond designated a.

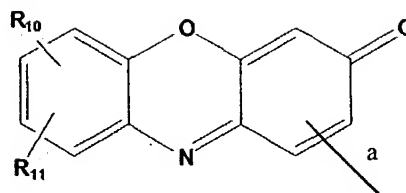
58. A method in accordance with Claim 57 where in the formula **Q** of the quencher moiety  $R_0$  is H,  $R_1$  is  $\text{NO}_2$  in the 4 position of the benzene nucleus,  $R_2$  is Cl in the 2 position of the benzene nucleus, and  $R_3$  and  $R_4$  are hydrogen and  $R_5$  is ethyl.

59. A method for hybridizing nucleic acids, comprising the steps of:  
 (a) providing a first nucleic acid and a second nucleic acid,  
 (b) incubating the nucleic acids under hybridization conditions, and  
 (c) identifying hybridized nucleic acids; wherein at least one of the nucleic acids comprises a **FL-nucleic-acid-Q** conjugate where **Q** is a quencher moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms, and

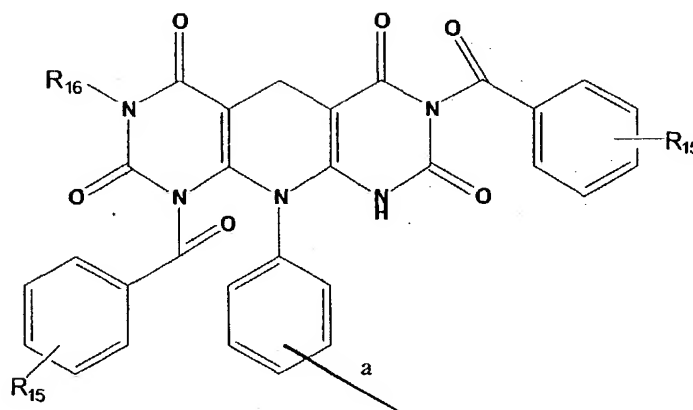
wherein FL is a fluorophore covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, said fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein  $R_8$  and  $R_9$  independently are H, halogen,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{C}(=\text{O})\text{NH}_2$ , or  $-\text{CN}$ ;  $-\text{OR}_{nn}$ ,  $-\text{SR}_{nn}$ ,  $-\text{OR}_{nn}$ ,  $-\text{NHR}_{nn}$ ,  $-\text{N}[\text{R}_{nn}]_2$  where  $\text{R}_{nn}$  is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

$R_{10}$  and  $R_{11}$  independently are H,  $-\text{CN}$ ,  $-\text{OR}_{12}$ ,  $-\text{N}(\text{R}_{12})_2$ , halogen,  $-\text{O}(\text{CH}_2)_n\text{CH}_3$ ,  $-(\text{CH}_2)_n\text{CH}_3$ ,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{C}(=\text{O})\text{NH}_2$ ,  $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$  where  $n=0$  to 5, or  $\text{R}_{12}$  is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

$\text{R}_{15}$  is H or alkyl of 1 to 10 carbons;

$\text{R}_{16}$  is alkyl of 1 to 10 carbons, and

the valence bond designated **a** symbolizes covalent attachment of the fluorophore to the linker.

**60.** A method in accordance with Claim 59 where the fluorophore has the formula designated FL-1.

61. A method in accordance with Claim 60 where  $R_8$  is  $OC(O)CH(CH_3)_2$  and  $R_9$  is H.

62. A method in accordance with Claim 59 where the fluorophore has the formula designated FL-2.

63. A method in accordance with Claim 62 where  $R_{10}$  is  $OC(O)CH(CH_3)_2$  and  $R_{11}$  is H.

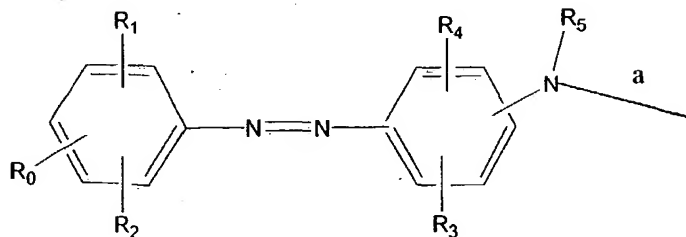
64. A method in accordance with Claim 59 where the fluorophore has the formula designated FL-3.

65. A method in accordance with Claim 64 where  $R_{15}$  is methyl and  $R_{16}$  is *n*-propyl.

66. A method for discriminating between polynucleotides which differ by a single nucleotide, the method comprising the following steps:

- (a) providing a polynucleotide comprising a target sequence,
- (b) providing at least two **FL-ODN-Q** conjugates, wherein ODN represents an oligonucleotide moiety, one of the at least two **FL-ODN-Q** conjugates has a sequence that is perfectly complementary to the target sequence and at least one other of the **FL-ODN-Q** conjugates has a single-nucleotide mismatch with the target sequence;
- (c) separately incubating each of the **FL-ODN-Q** conjugates with the polynucleotide under hybridization conditions; and
- (d) determining the hybridization strength between each of the **FL-ODN-Q** and the polynucleotide, wherein **FL** is a fluorophore moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms, and **Q** is a quencher moiety covalently attached to

the nucleic acid through a linker having the length of 0 to approximately 30 atoms, the quencher moiety having the structure



where  $R_0$ ,  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are independently -H, halogen,  $-O(CH_2)_nCH_3$ ,  $-(CH_2)_nCH_3$  where  $n = 0$  to 5,  $-NO_2$ ,  $-SO_3$ ,  $-N[(CH_2)_nCH_3]_2$  where  $n' = 0$  to 5 or  $-CN$ , and  $R_5 = -H$  or  $-(CH_2)_nCH_3$  where  $n'' = 0$  to 5, and where the quencher moiety is attached to the linker through the valence bond designated a.

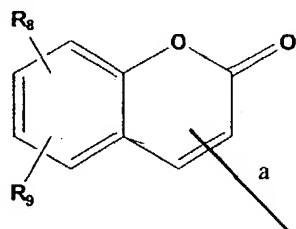
67. A method in accordance with Claim 66 where in the formula of the quencher moiety  $Q$   $R_0$  is H,  $R_1$  is  $NO_2$  in the 4 position of the benzene nucleus,  $R_2$  is Cl in the 2 position of the benzene nucleus, and  $R_3$  and  $R_4$  are hydrogen and  $R_5$  is ethyl.

68. A method for discriminating between polynucleotides which differ by a single nucleotide, the method comprising the following steps:

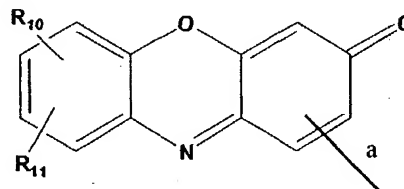
- (a) providing a polynucleotide comprising a target sequence,
- (b) providing at least two **FL-ODN-Q** conjugates, wherein ODN represents an oligonucleotide moiety, one of the at least two **FL-ODN-Q** conjugates has a sequence that is perfectly complementary to the target sequence and at least one other of the **FL-ODN-Q** conjugates has a single-nucleotide mismatch with the target sequence;
- (c) separately incubating each of the **FL-ODN-Q** conjugates with the polynucleotide under hybridization conditions; and
- (d) determining the hybridization strength between each of the **FL-ODN-Q** and the polynucleotide, wherein  $Q$  is a quencher moiety covalently



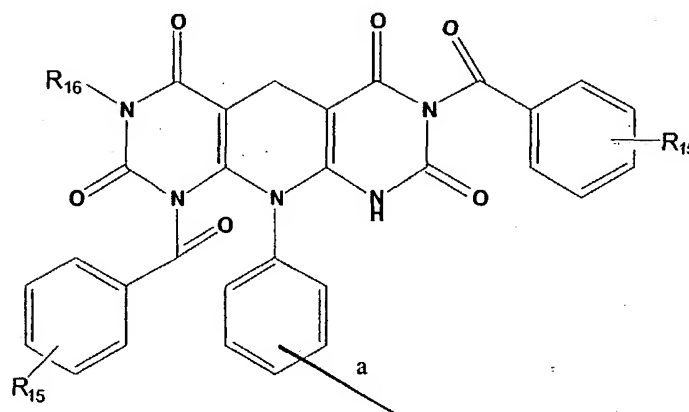
attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms, and **FL** is a fluorophore moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms,, and the fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein  $R_8$  and  $R_9$  independently are H, halogen,  $-NO_2$ ,  $-SO_3$ ,  $-C(=O)NH_2$ , or  $-CN$ ;  $-OR_{nn}$ ,  $-SR_{nn}$ ,  $-OR_{nn}$ ,  $-NHR_{nn}$ ,  $-N[R_{nn}]_2$  where  $R_{nn}$  is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

$R_{10}$  and  $R_{11}$  independently are H,  $-CN$ ,  $-OR_{12}$ ,  $-N(R_{12})_2$ , halogen,  $-O(CH_2)_nCH_3$ ,  $-(CH_2)_nCH_3$ ,  $-NO_2$ ,  $-SO_3$ ,  $-C(=O)NH_2$ ,  $-N[(CH_2)_nCH_3]_2$  where  $n = 0$  to 5, or  $R_{12}$  is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

$R_{15}$  is H or alkyl of 1 to 10 carbons;

$R_{16}$  is alkyl of 1 to 10 carbons, and  
the valence bond designated  $\Delta$  symbolizes covalent attachment of the  
fluorophore to the linker.

69. A method in accordance with Claim 68 where the fluorophore has  
the formula designated FL-1.

70. A method in accordance with Claim 69 where  $R_8$  is  
 $OC(O)CH(CH_3)_2$  and  $R_9$  is H.

71. A method in accordance with Claim 68 where the fluorophore has  
the formula designated FL-2.

72. A method in accordance with Claim 71 where  $R_{10}$  is  
 $OC(O)CH(CH_3)_2$  and  $R_{11}$  is H.

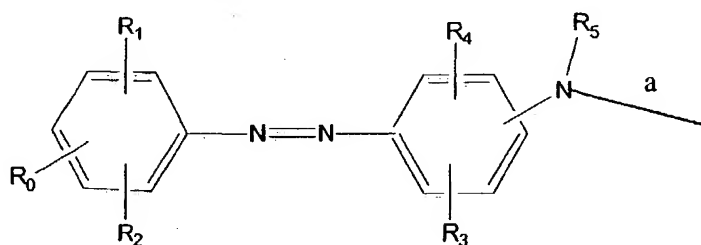
73. A method in accordance with Claim 68 where the fluorophore has  
the formula designated FL-3.

74. A method in accordance with Claim 73 where  $R_{15}$  is methyl and  $R_{16}$   
is *n*-propyl.

75. A method for hybridizing nucleic acids, comprising the steps of:  
(a) providing a first nucleic acid and a second nucleic acid,  
(b) incubating the nucleic acids under hybridization conditions, and  
(c) identifying hybridized nucleic acids;

wherein at least one of the nucleic acids comprises a **FL-nucleic-acid-Q-MGB** conjugate where **FL** is a fluorophore moiety covalently attached to  
the nucleic acid through a linker having the length of 0 to approximately 30  
atoms, MGB is minor groove binder moiety covalently attached to the ODN

moiety or to the quencher moiety through a linker having the length of 0 to approximately 30 atoms and **Q** is a quencher moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms, the quencher moiety having the structure

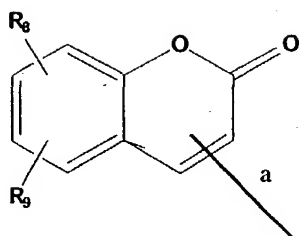


where  $R_0$ ,  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are independently -H, halogen,  $-O(CH_2)_nCH_3$ ,  $-(CH_2)_nCH_3$  where  $n=0$  to 5,  $-NO_2$ ,  $-SO_3$ ,  $-N[(CH_2)_{n'}CH_3]_2$  where  $n'=0$  to 5 or  $-CN$ , and  $R_5 = -H$  or  $-(CH_2)_{n''}CH_3$  where  $n''=0$  to 5, and where the quencher moiety is attached to the linker through the valence bond designated a.

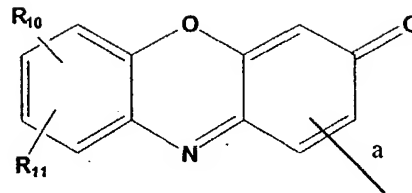
76. A method for hybridizing nucleic acids, comprising the steps of:

- (a) providing a first nucleic acid and a second nucleic acid,
- (b) incubating the nucleic acids under hybridization conditions, and
- (c) identifying hybridized nucleic acids;

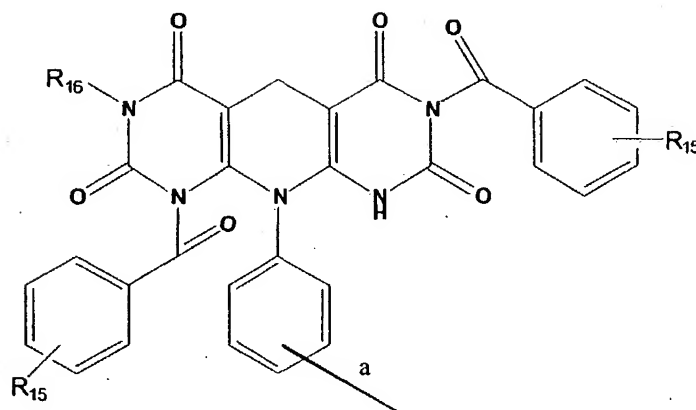
wherein at least one of the nucleic acids comprises a **FL-ODN-Q-MGB** conjugate where ODN is a nucleic acid or modified nucleic acid, MGB is minor groove binder moiety covalently attached to the ODN moiety or to the quencher moiety through a linker having the length of 0 to approximately 30 atoms, **Q** is a quencher moiety covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, and **FL** is a fluorophore moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms, and the fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein  $R_8$  and  $R_9$  independently are H, halogen,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{C}(=\text{O})\text{NH}_2$ , or  $-\text{CN}$ ;  $-\text{OR}_{nn}$ ,  $-\text{SR}_{nn}$ ,  $-\text{OR}_{nn}$ ,  $-\text{NHR}_{nn}$ ,  $-\text{N}[\text{R}_{nn}]_2$  where  $R_{nn}$  is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

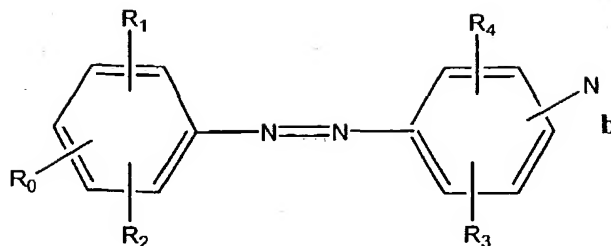
$R_{10}$  and  $R_{11}$  independently are H,  $-\text{CN}$ ,  $-\text{OR}_{12}$ ,  $-\text{N}(\text{R}_{12})_2$ , halogen,  $-\text{O}(\text{CH}_2)_n\text{CH}_3$ ,  $-(\text{CH}_2)_n\text{CH}_3$ ,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{C}(=\text{O})\text{NH}_2$ ,  $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$  where  $n=0$  to 5, or  $R_{12}$  is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

$R_{15}$  is H or alkyl of 1 to 10 carbons;

$R_{16}$  is alkyl of 1 to 10 carbons, and

the valence bond designated a symbolizes covalent attachment of the fluorophore to the linker; and

Q comprises a diazo moiety having the formula:

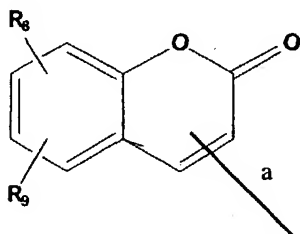


wherein covalent attachment to the linker is through the nitrogen atom designated as **b**.

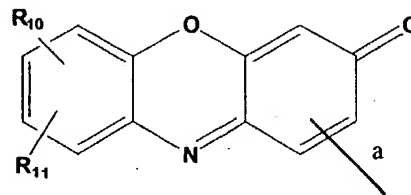
77. A method for hybridizing nucleic acids, comprising the steps of:

- (a) providing a first nucleic acid and a second nucleic acid,
- (b) incubating the nucleic acids under hybridization conditions, and
- (c) identifying hybridized nucleic acids;

wherein at least one of the nucleic acids comprises a **FL-ODN-Q-MGB** conjugate where ODN is a nucleic acid or modified nucleic acid, MGB is minor groove binder moiety covalently attached to the ODN moiety or to the quencher moiety through a linker having the length of 0 to approximately 30 atoms, **Q** is a quencher moiety covalently attached to the ODN through a linker having the length of 0 to approximately 30 atoms, and **FL** is a fluorophore moiety covalently attached to the nucleic acid through a linker having the length of 0 to approximately 30 atoms,, and the fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



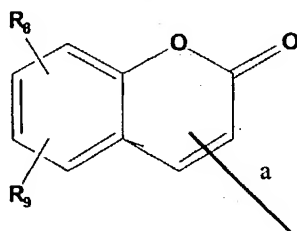
FL-1



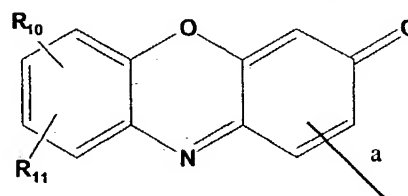
FL-2



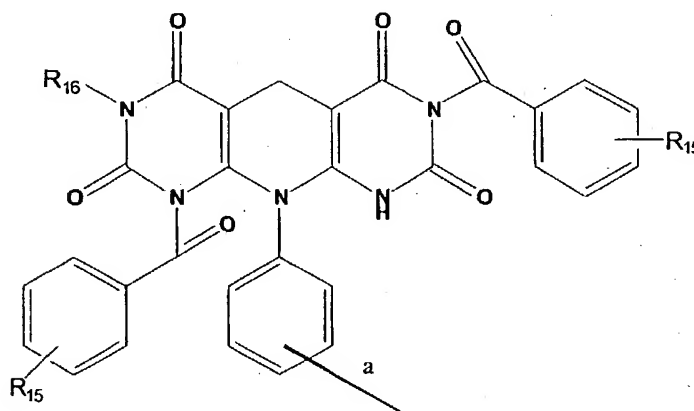
to approximately 30 atoms,, and the fluorophore moiety having the structure selected from the group designated FL-1, FL-2 and FL-3,



FL-1



FL-2



FL-3

wherein  $R_8$  and  $R_9$  independently are H, halogen,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{C}(=\text{O})\text{NH}_2$ , or  $-\text{CN}$ ;  $-\text{OR}_{nn}$ ,  $-\text{SR}_{nn}$ ,  $-\text{OR}_{nn}$ ,  $-\text{NHR}_{nn}$ ,  $-\text{N}[\text{R}_{nn}]_2$  where  $\text{R}_{nn}$  is independently H, an alkyl group of 1 to 10 carbons or an alkanoyl group of 1 to 10 carbons;

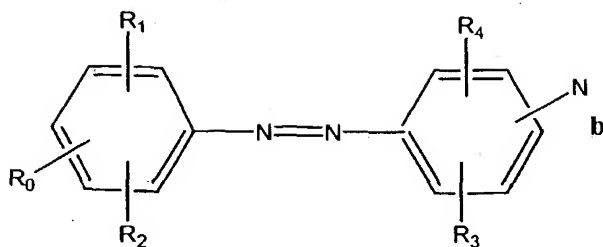
$R_{10}$  and  $R_{11}$  independently are H,  $-\text{CN}$ ,  $-\text{OR}_{12}$ ,  $-\text{N}(\text{R}_{12})_2$ , halogen,  $-\text{O}(\text{CH}_2)_n\text{CH}_3$ ,  $-(\text{CH}_2)_n\text{CH}_3$ ,  $-\text{NO}_2$ ,  $-\text{SO}_3$ ,  $-\text{C}(=\text{O})\text{NH}_2$ ,  $-\text{N}[(\text{CH}_2)_n\text{CH}_3]_2$  where  $n = 0$  to 5, or  $\text{R}_{12}$  is alkyl of 1 to 10 carbons alkanoyl of 1 to 10 carbons,;

$\text{R}_{15}$  is H or alkyl of 1 to 10 carbons;

$\text{R}_{16}$  is alkyl of 1 to 10 carbons, and

the valence bond designated **a** symbolizes covalent attachment of the fluorophore to the linker; and

**Q** comprises a diazo moiety having the formula:



wherein covalent attachment to the linker is through the nitrogen atom designated as **b**.